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(71) Applicant *(for all designated States except US)*: JUNGVID, Hans [SE/SE]; Thorstorp, P O Box 134, S-430 20 Veddige (SE).

(71)(72) Applicants and Inventors: VON DER DECKEN, Alexandra [SE/SE]; Docentbacken 3, S-104 05 Stockholm (SE). OLIN. Thomas [SE/SE]; Enbacken 33, S-183 46 Täby (SE).

(74) Agent: INGER, Lars, Ulf, Bosson; L&U Inger Patentbyrå AB, Garvaregatan 12, S-262 63 Ängelholm (SE).

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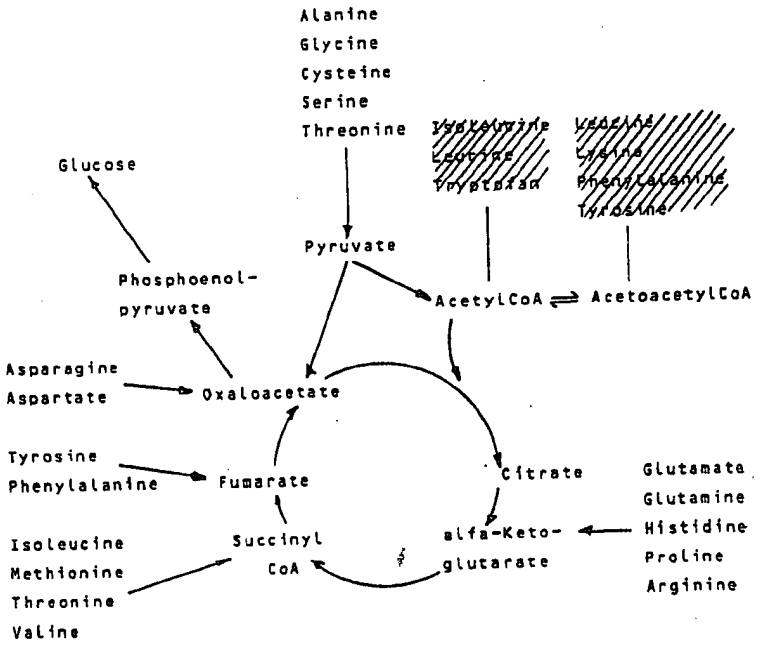
(54) Title: FOOD AND FEEDSTUFF

(57) Abstract

The present invention relates to a process for reducing the excretion of nitrogen from humans and animals while maintaining formation of proteins, as well as a food and feedstuff for humans and animals comprising keto acids, whereby ingoing protein is replaced by alpha ketoglutaric acid; a combination of alpha keto glutaric acid and pyruvic acid; oxaloacetate or oxaloacetate in combination with alpha keto glutaric acid or pyruvate or another keto acid according to the formula R-CO-COOX, wherein R denotes a part of a non-essential amino acid and X denotes hydrogen, alkali metal, alkaline earth metal or organic base or non-toxic precursors or intermediates of these, whereby the keto acid(-s) is (are) present in an amount of 1 to 20 % by weight, preferably 1-10 % by weight of the protein dry matter present.



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FOOD AND FEEDSTUFF

DESCRIPTION

Technical field

The present invention relates to a food and feedstuff for humans and animals comprising keto acids.

The object of the present invention is to obtain a food and feedstuff, which having a reduced content of proteins still might fulfil the demand of the animals as to access to protein forming and energy providing substances. Furthermore, the feedstuff shall fulfil the demand for access to biomolecules deriving from non-essential amino acids. The invention further aims to reduce the excretion of nitrogen from the organism.

Background of the invention

It is well known that water living carnivores, such as salmon, and land based carnivores, such as mink, live under natural conditions, on a diet consisting of 30 to 55 % of proteins (90 % DS). The choice of food is depending on live phase and specie. A great deal of this protein can be oxidized to form energy providing compounds, such as ATP (adenosine triphosphate), which results in excretion of nitrogen to the environment. At an intense cultivation of e.g. fish and mink this might cause an environmental problem.

During natural conditions omnivores live on a diet consisting of 15 to 30 % of proteins (90 % DS). During certain conditions this protein might be used for providing energy.

The most energy demanding tissue in the body is the muscles, which obtains its carbohydrate energy from the food directly via the blood or from the liver via the blood in the form of glucose. Glucose is formed in the liver from inter alia decomponents of the proteins of the muscles. The compounds which hereby form glucose are substantially alanine and its corresponding keto acid. It has also been shown that the corresponding keto acids of some essential amino acids, such as alpha keto

isocaproat (KIC), may be precursors of amino acids, e.g. KIC of leucine.

It is previously known from EP-A1-0 237 061 and EP-A1-0 237 959 to add alpha-ketoisocaproate (KIC) to egg producing hen to increase the quantity of eggs and to improve the quality of the eggs produced, to milk producing domestic animals to increase the quantity of milk and to improve the quality of the milk produced. KIC is thereby added in an amount of 0.01 to 2 % by weight, preferably 0.05 to 1.5 % by weight calculated on the dry weight of the feed-stuff composition. KIC is the only pure ketogenic keto acid of the keto acids formed from the amino acids.

From US-A-4,548,937, and US-A-4,645,764 it is known to administer to humans and animals a therapeutically effective amount of pyruvate. At the administration of this compound a reduced gain in weight is obtained, decreased fat depots, and an increased amount of glycogen in the liver. None of these patents has disclosed an increased efficiency of the protein of the food.

It is previously known from US-A-4,361,570 to add a pyridoxine-alpha-ketoglutarate (PAK) to a feed-stuff in order to treat hyperlacticacidemia, whereby PAK is a conjugate between pyridoxine and alpha-ketoglutarate. This molecule can not be compared chemically with free alpha-ketoglutarate.

Amino acids which are not used for the synthesis of proteins can not be stored in the organism. They are then reformed to mainly fat or via keto acids, to carbohydrates. Both these macromolecules can take part in the energy metabolism. The metabolic interaction (cf. FIG. 1) between liver and muscle shows the importance of carbon bodies from amino acids. KIC is the only pure ketogenic keto acid of the keto acids formed from amino acids. Alpha-ketoglutarate (KG) and pyruvate are classified as glycogenic keto acids. The latter keto acids have a

central position in said interaction. The catabolism of KIC is schematically evident from FIG. 2. In FIG. 3 the connection between the different amino acids and their metabolic products of the citric acid cycle is shown.

KG is an important part of the metabolism which is examplified by the following:

(i) NH_4^+ is coupled to amino acids via the reaction NH_4^+ + KG + NADPH ==== Glutamate + $NADP^+$ + H_2O
Glutamate provides the alpha amino group in the synthesis of most non-essential amino acids;

(ii) the carbon skeletons present in the synthesis of non-essential amino acids are all intermediates of the glucolysis, the pentose-monophosphate shunt or the citric acid cycle and may hereby be derived from pyruvate or KG (cf FIG. 5)

(iii) at physiological stress, too large energy draft, reproductiocn conditions and other low energy situations, the oxidation of amino acids increases whereby a deamination with KG or PY as amino receptors belongs to the starting processes (cf. FIG. 6);

(iv) at the synthesis of collagen KG is essential to the hydroxylation of proline.

Pyruvate is a centre of the metabolism (cf. FIG. 4), where the following steps can be distinguished:

(i) the formation of lactate to provide the glucolysis with NAD^+ during aerobic conditions;

(ii) formation of acetyl-CoA for the synthesis of fatty acids or digestion in the citric acid cycle;

(iii) a high concentration of acetyl-CoA increases the formation of oxaloacetate, which either provides the citric acid cycle with intermediates (at a low concentration of ATP (adenosine triphosphate) or becomes a substrate in the gluconeogenesis (at high ATP concentrations);

(iv) at the gluconeogenesis pyruvate might possibly contribute to the pentose-monophosphate shunt, where NADPH is formed (essential in several anabolic processes, i.a. the synthesis of the non-essential amino acids) and ribose-5-phosphate, which is a substrate at the synthesis of nucleotides;

(v) the formation of alanine as well as other non-essential amino acids where pyruvate contributes with the carbon skeleton.

Description of the present invention

It has now surprisingly been shown possible, with a maintained formation of proteins, to be able to reduce the excretion of nitrogen from animals and man by replacing a part of the protein of food and feedstuffs by means of the present invention. This is characterized in that the ingoing protein is replaced by alpha keto glutaric acid; a combination of alpha keto glutaric acid and pyruvic acid; oxaloacetate or oxaloacetate in combination with alpha keto glutaric acid or pyruvate or another keto acid according to the formula R-CO-COOX, wherein R denotes a part of a non-essential amino acid and X denotes hydrogen, alkali metal, alkaline earth metal or organic base or non-toxic precursors or intermediates of these.

The keto acids above can generally be defined by the formula R-CO-COOX, wherein R denotes a part of a non-essential amino acid and X denotes hydrogen, alkali metal, alkaline earth metal or organic base or non-toxic precursors or intermediates of such keto acids. These compounds will in the following for the reason of simplicity be called keto acids. These keto acids are present in an amount of 1 to 20 % by weight, preferably 1 to 10 % by weight, of the dry substance of the ingoing proteins.

Further characteristics will be evident from the accompanying claims.

Administration can be made to carnivores, omnivores and herbi-

vores to reduce, in applicable cases, the excretion of nitrogen but also to achieve a protein saving effect.

By means of the present invention thus substrate in the form of keto acids of non-essential amino acids is supplied for the formation of proteins or other biomolecules deriving from these amino acids. The keto acids, which can metabolize, during aerobic conditions, in the mitochondria of the cells, give raise to energy providing and reducing compounds. Both these types of compounds are essential in active life processes. In the mitochondria intermediates are formed during the metabolism of keto acids, i.a. succinyl-CoA. Succinyl-CoA is present in the synthesis of the porfyrine molecule in haemoglobin and myoglobin. When keto acids are added to the organism a protein saving effect is obtained as the organism can use keto acids for non-essential amino acids in stead of using amino acids for other purposes than the formation of protein. Thereby the efficiency of the protein added increases. KG, one of the above described keto acids has a central position in the binding of NH_4^+ and later in the synthesis of non-essential amino acids. Different keto acids of non-essential amino acids are furthermore a more effective substrate than the amino acids themselves as no deamination needs to take place and the energy demanding excretion of nitrogen from the organism decreases. As evident from the Example 1 below, where fish spawns having obtained a feedstuff being supplemented with 10 % keto acids, reduces the excretion of nitrogen with more than 20 %.

The present invention can also be used for parenteral nutrition at catabolic situations, e.g. at large trauma, such as burn damages etc. but also at kidney damages.

During the reproductive phase when the fish mainly utilizes its muscle proteins added keto acids in stead be utilized, e.g. pyruvate, optionally in combination with KG. In that way the otherwise great loss of muscle protein can be reduced. Furthermore, the excretion of nitrogen is reduced.

The present invention will in the following be described more in detail with reference to the Examples given, however, without being restricted thereto.

Example 1

Growth test.

1-summer spawns of salmon were fed during 5 weeks with partly a control feedstuff intended for very young salmon, partly a feedstuff according to the present invention based upon the same raw materials as the control feedstuff but moreover containing 5 % of pyruvate Na-salt, and 5 % alpha keto glutaric acid (KG), whereby 10 % of the protein in the form of fish meal present in the control feedstuff had been replaced by keto acids. The test was carried out in such a way that 500 spawns in one trough obtained the control feedstuff and 500 spawns in another trough obtained a feedstuff according to the present invention. The spawn of salmon had an average weight of about 4 g each at the beginning of the test. Growth, nitrogen excre-tion, total analysis and protein analysis were carried out. Macroscopic investigation of the spawns was carried out as well.

Composition of the feedstuff	Control	Test feedstuff
Vitamin and mineral premix	2.0 %	2.0 %
Binding agent, lignosulphate	2.0 %	2.0 %
Wheat, boiled in an autoclave	18.8 %	18.8 %
Soya, finely ground	4.0 %	4.0 %
Blood meal	2.0 %	2.0 %
Fish meal, whole meal	63.2 %	53.2 %
Lodda oil, lecithin	10.0 %	10.0 %
Alpha keto glutaric acid	-	5.0 %
Na-pyruvate	-	5.0 %
Total	100.0 %	100.0 %

Feedstuff analysis	Control	Test feedstuff
N-content	9.46 %	7.90 %
Cysteine	5.8 g/kg	4.7 g/kg
Methionine	17.0	13.5
Asparginic acid	56.6	44.9
Threonine	25.2	20.4
Serine	26.2	21.0
Glutamic acid	89.8	75.2
Proline	27.4	21.0
Glycine	36.8	28.3
Alanine	38.1	30.4
Valine	33.6	27.0
Isoleucine	26.0	20.4
Leucine	46.3	37.1
Tyrosine	19.5	15.3
Phenyl alanine	24.2	20.0
Histidine	14.7	12.0
Lysine	43.9	34.3
Arginine	34.5	27.6
Hydroxyproline	7.5	3.7
Sum	572.9	456.9
Ammonia	9.0	7.2
Total	581.9	464.1

The keto acids of the test feedstuff thus were 10 % of the feedstuff and substituted 20 % of the amino acids and thus reduced the nitrogen content with about 16 %. Amino acid analyses of the feedstuff and the fish are carried out in accordance with official EEC-method.

Analysis, spawns 100 % DS	Control	Test feedstuff
N-content	8.85 %	8.86 %
Cysteine	6.1 g/kg	5.8 g/kg
Methionine	17.7	15.5
Asparginic acid	53.8	51.1
Threonine	24.3	22.9
Serine	23.7	22.5
Glutamic acid	87.3	84.7
Proline	23.5	23.0
Glycine	38.6	38.1
Alanine	35.7	34.2
Valine	38.9	27.7
Isoleucine	23.2	23.2
Leucine	41.0	39.5
Tyrosine	18.5	17.9
Phenyl alanine	24.2	23.2
Histidine	13.2	12.8
Lysine	43.9	43.5
Arginine	32.4	32.0
Sum	534.7	517.4
Ammonia	8.2	7.3
Total	542.9	524.7

The results are mean values of three analyses based on 50 spawns each. The differences between the groups are non-significant.

Nitrogen excretion tests

In order to determine the excretion of nitrogen 10 spawns of each group were allowed to live in a closed trough filled with oxygenised water. The spawns lived in this environment during 90 minutes, whereupon they were killed, weighed and dissected. All spawns had their stomachs as well as intestines well filled with feedstuff. The excretion of NH_3 and NH_4^+ were analysed in water samples from the two troughs in such a way that the time

differences between feeding, killing and final sampling was uniform. Ammonia was analysed in accordance with Parsons et al, A Manual of Chemicaland Biological Methods for Seawater Analysis, Pergamon Press, pages 15-16 (ISBN-0-08-030288-2). The test which was carried out as a final of an earlier growth test was carried out when the fishes were about 20 weeks of age. No difference in mortality between control and test group could be noted during the added test periods.

Results

Group of fishes	Weight (g+SEM)	Excretion of nitrogen ($\mu g\ NH_4^+/g\ BW^{-1} \times 1\ hr^{-1}$)
Control feedstuff	3.75±0.15	8.00±0.69
Test feedstuff	3.77±0.21	5.71±0.37

The difference in excretion of nitrogen between the test groups is 28% and statistical significance in accordance with students t-test is $n=20$, $p \leq 0.01$.

Macroscopic analysis of the spawns.

Dissection of the spawns showed no difference between the groups with regard to liver status, stomach-intestinal filling and accumulation of visceral fat.

The spawns of the control group had at the beginning of the test an average weight of 3.40 g and at the end of the test an average weight of 3.95 g, while the group fed a feedstuff according to the present invention had an average weight of 3.56 g at the beginning of the test, and 4.11 g at the end of the test. The growth was thus equal.

Example 2

In a test using recently veined rats according to Eggum (Eggum, B.O.; A Study of Certain Factors Influencing Protein Utilization in Rats and Pigs, National Institute of Animal Science, 1973), p.406) where a control feedstuff consisting of cereals,

other carbohydrates, fish protein, fat, mineral, and vitamins was compared with a test feedstuff where 0.6 % by weight of pyruvate and 0.8% by weight of alfa-ketoglutarate was allowed to replace 1.4% by weight of fish protein and where 0.15% by weight of soya oil was added to the test feedstuff in order to compensate for the difference in fat content appearing by the reduced content of fish protein, the following results were obtained:

Table 1

	Control group	Test group
Fecal digestibility (TD), %	91.2 (1.6)	92.4 (1.0)
Biological value (BV), %	92.8 (1.1)	94.6 (0.5)
Net protein utilization (NPU), %	84.7* (1.9)	87.4* (0.7)
Growth, grams	13.8 (2.3)	13.6 (1.7)
Protein of feedstuff, % DS	10.39	9.16

Standard deviation within parenthesis
* Significant difference according to Student's t-test $p<0.02$

The test, which was carried out in such a way that the total amount of feedstuff (g DS) was equal to the two groups of animals, showed no significant growth differences in spite of the fact that 1.4 % by weight of the protein in the control feedstuff had been replaced by keto acids.

The net protein utilization which shows the animals' possibility of utilizing the protein added for building up a protein of its own shows as evident from Table 1, significant differences, mainly depending on better biological value (BV) of the test group. The protein improving effect is explained by the addition of, in this case, a combination of alfa-keto glutarate and pyruvate.

Macroscopical investigations of liver, kidneys, and other internal organs showed, moreover, no differences between the

groups of animals.

At a composition of a feedstuff according to the present invention where large parts of the protein have been replaced by keto acids in accordance with above, the amount of salt should be restricted in order to avoid electrolytical disturbances. Thus other salts than sodium salts of the keto acids might be present, such as potassium, magnesium, and calcium salts. Free acids may be used as well.

CLAIMS

1. Process for reducing the excretion of nitrogen from humans and animals while maintaining formation of proteins, characterized in that one replaces ingoing protein by alpha ketoglutaric acid; a combination of alpha keto glutaric acid and pyruvic acid; oxaloacetate or oxaloacetate in combination with alpha keto glutaric acid or pyruvate or another keto acid according to the formula R-CO-COOX, wherein R denotes a part of a non-essential amino acid and X denotes hydrogen, alkali metal, alkaline earth metal or organic base or non-toxic precursors or intermediates of these, whereby the keto acid (-s) is (are) present in an amount of 1 to 20 % by weight, preferably 1-10 % by weight of the protein dry matter present.

2. Food and feedstuff for humans and animals comprising keto acids, characterized in that ingoing protein is replaced by alpha ketoglutaric acid; a combination of alpha keto glutaric acid and pyruvic acid; oxaloacetate or oxaloacetate in combination with alpha keto glutaric acid or pyruvate or another keto acid according to the formula R-CO-COOX, wherein R denotes a part of a non-essential amino acid and X denotes hydrogen, alkali metal, alkaline earth metal or organic base or non-toxic precursors or intermediates of these, whereby the keto acid (-s) is (are) present in an amount of 1 to 20 % by weight, preferably 1-10 % by weight of the protein dry matter present.

3. Food and feedstuff according to claim 2, characterized in that ingoing protein has been replaced by alfa-keto glutaric acid.

4. Food and feedstuff according to claim 2, characterized in that two keto acids are mixed in the weight relationship 25:75 to 75:25, preferably 40:60 to 60:40.

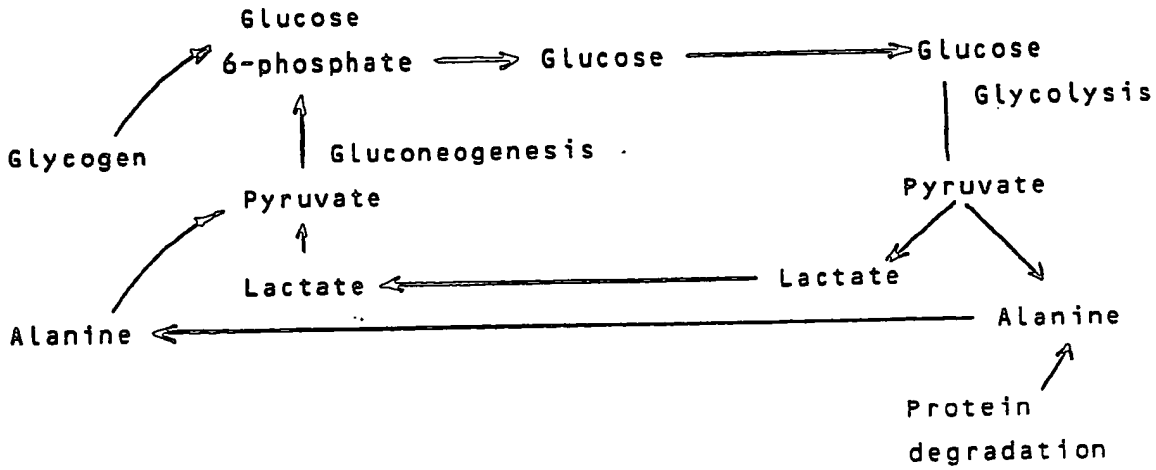
5. Food and feedstuff according to claim 2, characterized in that pyruvic acid and alfa-keto glutaric acid are present as keto acids.

6. Food and feedstuff according to claim 2, characterized in that oxaloacetate is present as keto acid.

7. Food and feedstuff according to claim 2, characterized in that oxaloacetate and alfa-ketoglutaric acid are present as keto acids.

8. Food and feedstuff according to claim 2, characterized in that oxaloacetate and pyruvic acid are present as keto acids.

9. Food and feedstuff according to claims 2 to 8, characterized in that the keto acids are present in the form of an acid and/or salt of alkali metal, alkaline earth metal or organic base.


Glucose
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Glucose
Glucose
Glycolysis
Glycogen
Gluconeogenesis
Pyruvate
Pyruvate
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Lactate
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Alanine
Protein
degradation
LIVER
MUSCLE

FIG. 1

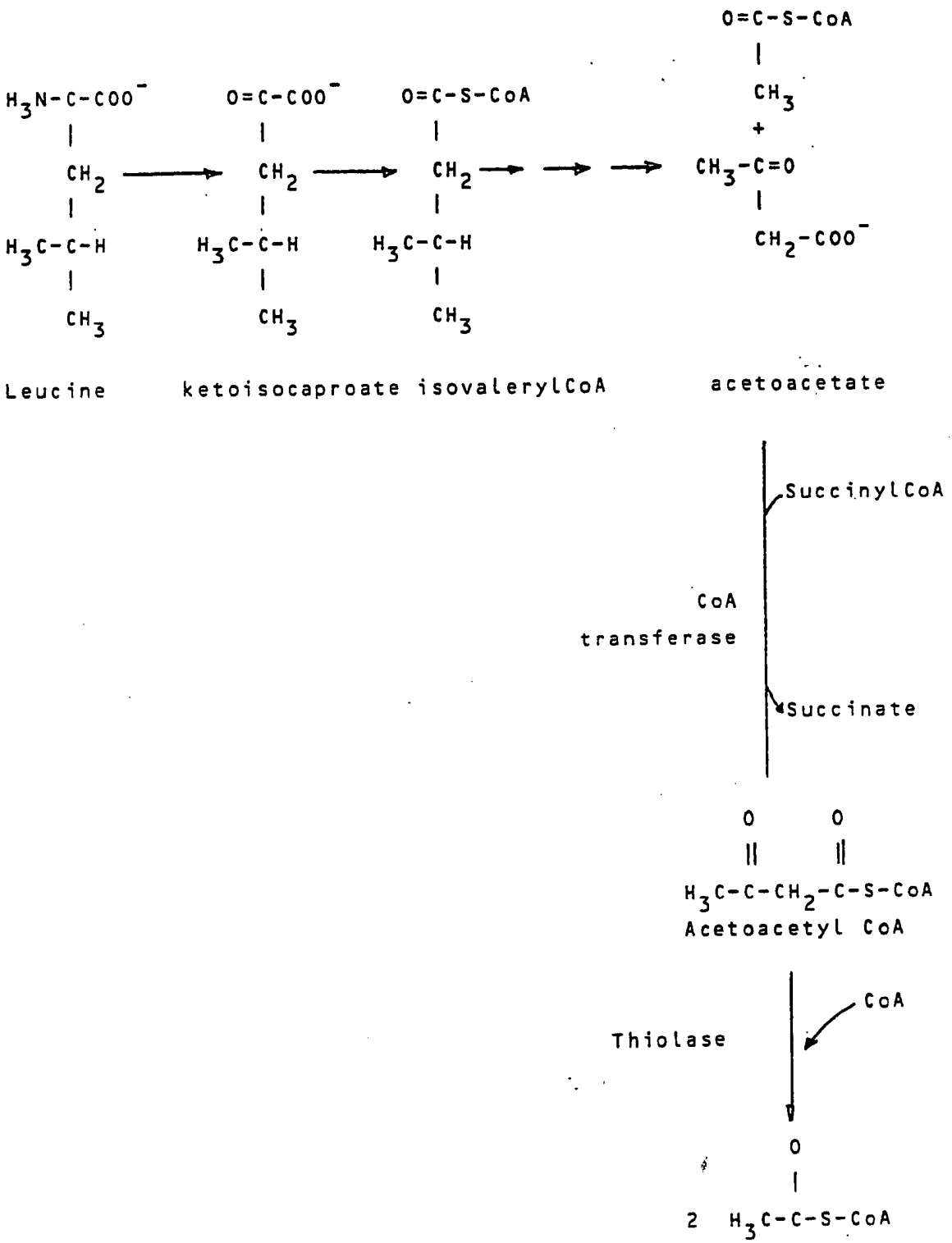
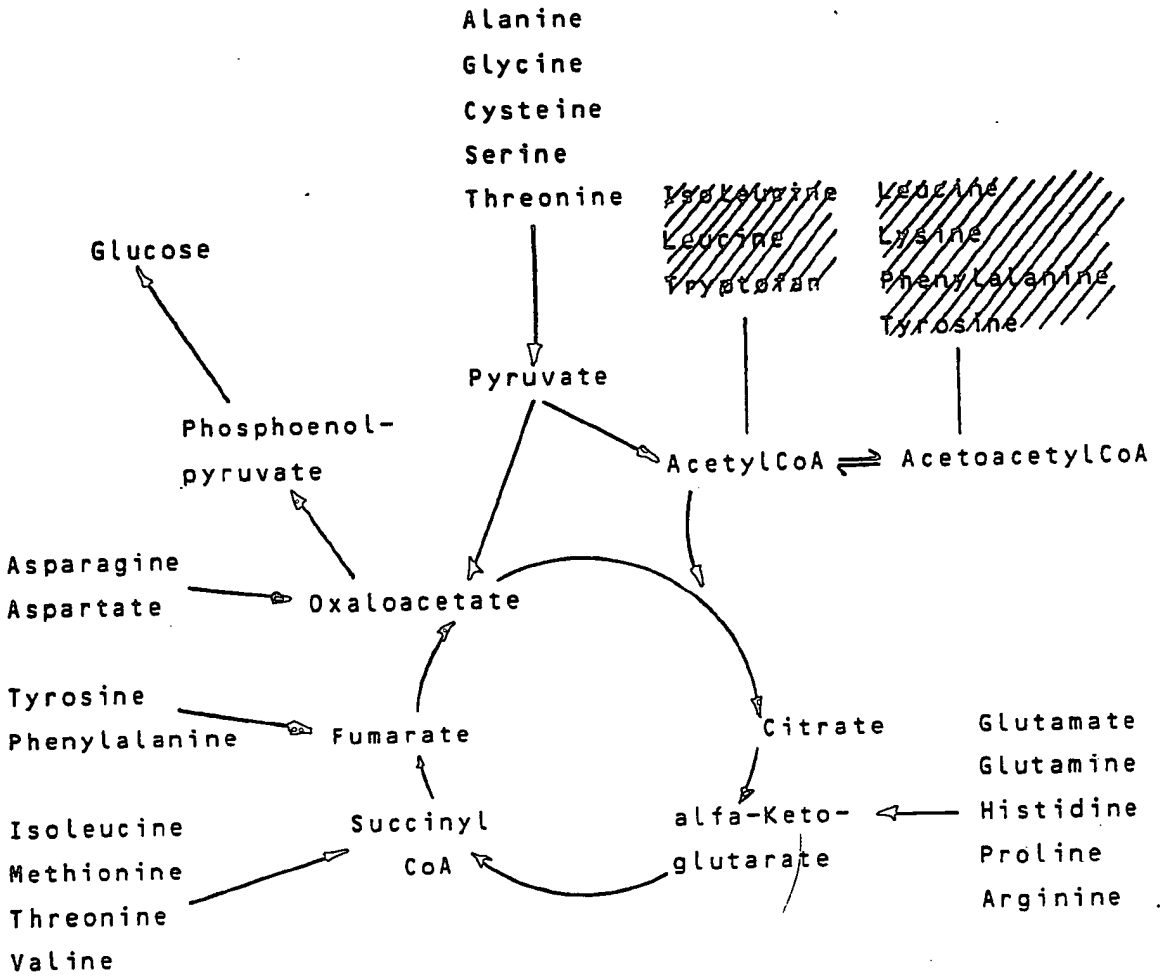

Leucine
ketoisocaproate
isovalerylCoA
acetoacetate
SuccinylCoA
CoA
transferase
Succinate
Acetoacetyl CoA
Thiolase
CoA
2

FIG. 2



= Ketogenic amino acids

FIG. 3

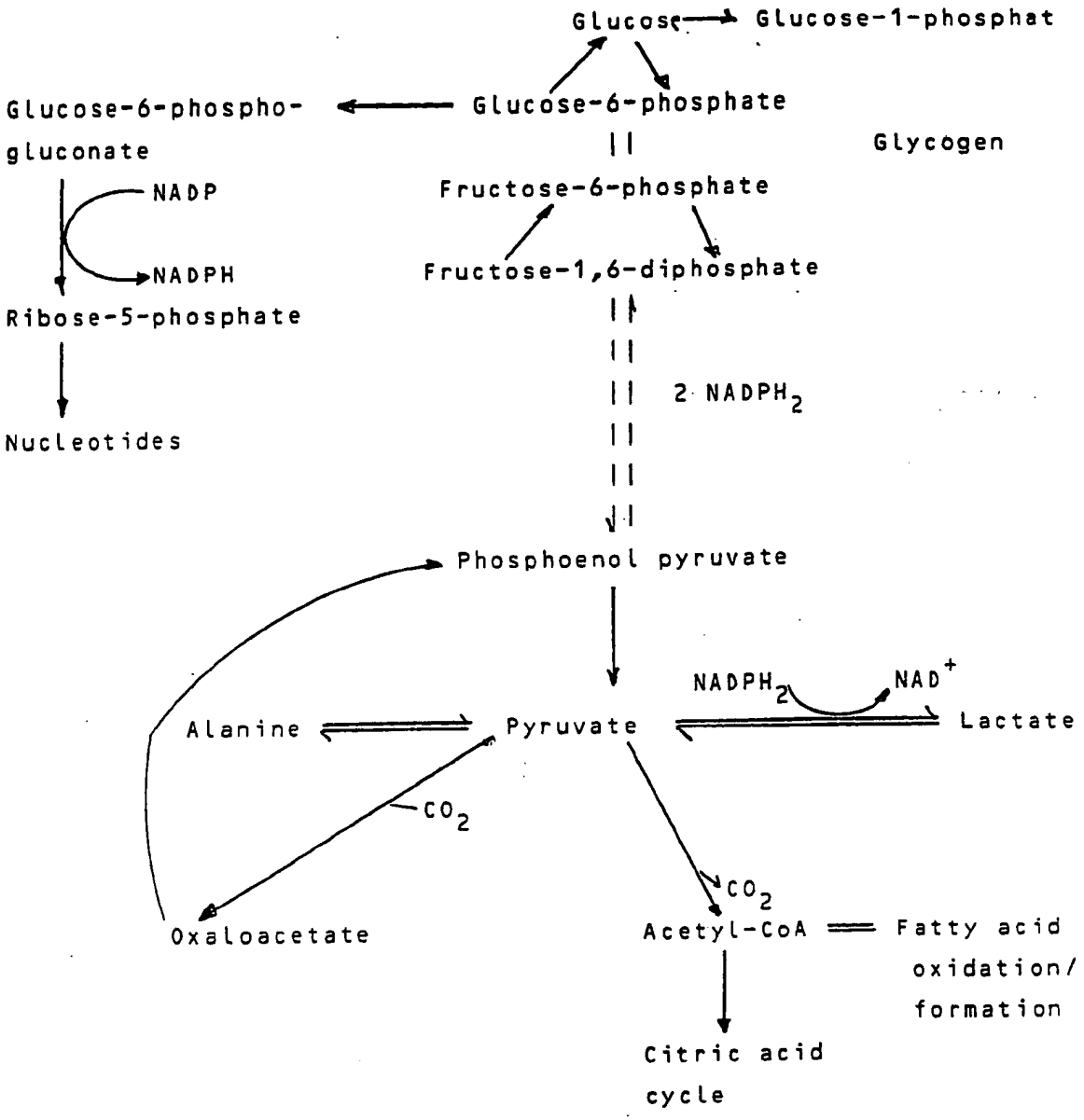

Glucose
Glucose-1-phosphat
Glucose-6-phospho-
gluconate
Glucose-6-phosphate
Glycogen
NADP
NADPH
Fructose-6-phosphate
Fructose-1,6-diphosphate
Ribose-5-phosphate
2 NADPH2
Nucleotides
Phosphoenol pyruvate
NADPH2
NAD+
Alanine
Pyruvate
Lactate
CO2
CO2
Oxaloacetate
Acetyl-CoA
Fatty acid
oxidation/
formation
Citric acid
cycle

FIG. 4

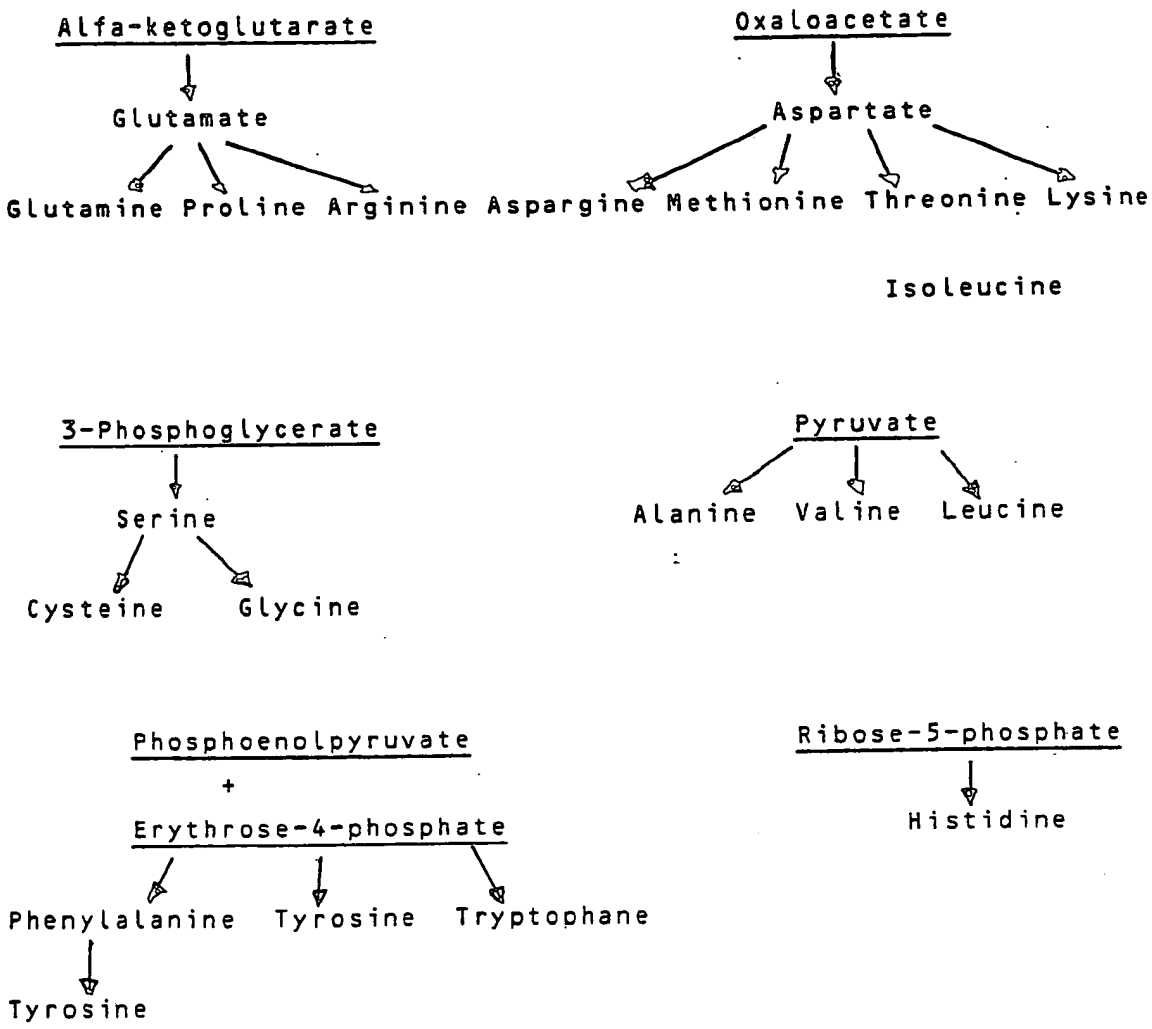

Alfa-ketoglutarate
Glutamate
Glutamine
Proline
Arginine
Oxaloacetate
Aspartate
Aspargine
Methionine
Threonine
Lysine
Isoleucine
3-Phosphoglycerate
Serine
Cysteine
Glycine
Pyruvate
Alanine
Valine
Leucine
Phosphoenolpyruvate
+
Erythrose-4-phosphate
Phenylalanine
Tyrosine
Tryptophane
Tyrosine
Ribose-5-phosphate
Histidine

FIG. 5

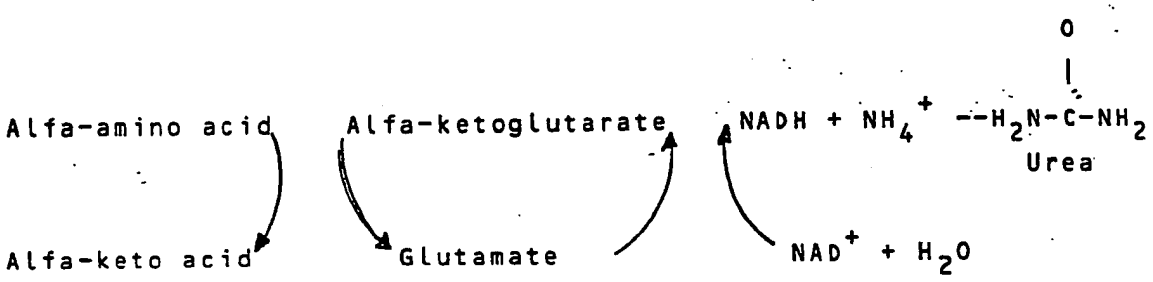

Alfa-amino acid
Alfa-keto acid
Alfa-ketoglutarate
Glutamate
NADH + NH4+
NAD+ + H2O
O
H2N-C-NH2
Urea

FIG. 6

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 89/00707

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 23 L 1/30, A 23 K 1/16

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC5	A 23 L; A 23 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	US, A, 4 744 990 (R.W. BRAGDON ET AL.) 17 May 1988, see the whole document --	2-9
A	US, A, 4 100 161 (M. WALSER) 11 July 1978, see the whole document --	2-9
A	US, A, 4 228 099 (M. WALSER) 14 October 1980, see the whole document --	2-9
A	US, A, 4 100 293 (M. WALSER) 11 July 1978, see the whole document --	2-9
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* Special categories of cited documents: [14]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25th January 1990	1990 -02- 15
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Inga-Karin Petersson

FURTHER INFORMATION C NTINUED FR M THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 1 because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treatment of the human or animal body by therapy [PCT Rule 39(iv)]

2. [ ] Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- [ ] The additional search fees were accompanied by applicant's protest.
- [ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/SE 89/00707

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.

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